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(71) Applicant *(for all designated States except US)*: GLAXO GROUP LIMITED [GB/GB]; Glaxo House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WELLS, Timothy, Nigel, Carl [GB/CH]; Glaxo Institute for Molecular Biology S.A., 14, chemin de Aulx, CH-1228 Plan-les-Ouates (CH). POWER, Christine, Anna [GB/CH]; Glaxo Institute for Molecular Biology S.A., 14, chemin des Aulx, CH-1228 Plan-les-Ouates (CH).

(74) Agents: MARTIN, Paul, White et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).

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(54) Title: A CHEMOKINE RECEPTOR ABLE TO BIND TO MCP-1, MIP-1 ALPHA AND/OR RANTES. ITS USES

(57) Abstract

A chemokine receptor binds to MCP-1, MIP-1 α and/or RANTES. It can be used in screening for agents which act as antagonists to MCP-1, MIP-1α and/or RANTES. Such agents may be useful in treating various disorders, including allergies, atheromas and diseases mediated by viruses. They may also be useful in preventing graft rejection and in protecting stem cells from potentially damaging effects of chemotherapy.

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A chemokine receptor able to bind to MCP-1, MIP-1 alpha and/or RANTES. Its uses.

The present invention relates to chemokine receptors.

Chemokines are a growing family of chemotactic cytokines, which have been implicated to play a role in the recruitment and activation of cells (Oppenheim, J.J. *et al.*, Ann Rev Immunol., 9 617-48, (1991), Schall, T.J., Cytokine, 3 165-183, (1991)). They are primarily responsible for the activation and recruitment of leukocytes, but not exclusively so. Further analysis of this superfamily of proteins has shown that it can be divided up into two further subfamilies of proteins. These have been termed CXC or α-chemokines, and the CC or β-chemokines based on the spacings of two conserved cysteine residues near to the amino terminus of the proteins.

To date two receptors have been identified for the CC chemokine family. The first, which is receptor primarily for MIP-1α (Macrophage inflammatory polypeptide-1α) and RANTES (Raised on Activation, Normal T-cell derived and Secreted) has been described previously (Gao, J.L. *et al.*, J. Exp. Med., **177** 1421-7 (1993), Neote, K. *et al.*, Cell **72** 415-25 (1993)). The second CC-chemokine receptor which has been recently described is for MCP-1 (monocyte chemotractant protein-1) Charo I. *et al.*, Proc. Natl. Acad. Sci. USA **91** 2752-2756 (1994). More recently, another receptor US28, expressed by the human cytomegalovirus, has been shown to be a receptor for RANTES, MIP-1α, and MCP-1 (Gao, J.L. and Murphy P.M., J. Biol. Chem. **269**, 28539-28542 (1994)). All three receptors are of the seven transmembrane alpha helical segment type, and are expressed into the membranes of cells.

However there remains a need to identify hitherto undisclosed chemokine receptors and to characterise them in order to develop a more complete picture of the structure and function of chemokine receptors.

According to the present invention there is provided a chemokine receptor having the amino acid sequence shown in Fig 3.

This receptor is preferably capable of binding MCP-1, MIP-1α and RANTES. It may be important in basophil and T-cell function.

It can be used to screen for pharmaceutically active agents. The present invention therefore includes within its scope such agents (which may or may not be proteins). They may be provided in a pharmaceutical composition together with a pharmaceutically acceptable carrier.

Such a composition is within the scope of the present invention. It may be prepared by admixing the carrier with the pharmaceutically active agent under sterile conditions. The pharmaceutical composition may be provided in unit dosage form. It may be present as part of a kit including instructions for use.

The pharmaceutical composition may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route.

A receptor of the present invention can be used to screen for agents useful in treating allergies e.g. asthma,

atopic dermatitis, rhinitis, hay fever, eczema or food allergies. It may also be useful in screening for agents useful in treating conjunctivitis. MCP-1, MIP-1α and RANTES all bind to the receptor of the present invention and are capable of causing histamine release from basophils. An agent which blocks this binding may thereby prevent or reduce the release of histamine from basophils (i.e. act antagonistically to MCP-1, MIP-1α or RANTES). Such agents may be variants of MCP-1, MIP-1α or RANTES (in which one or more amino acids are deleted, substituted or inserted relative to MCP-1, MIP-1α or RANTES), although this is not essential.

It may also be involved in the activation of T-lymphocytes, a common characteristic of immune and other inflammatory states.

The binding of agents to the receptor of the present invention can be assayed by suitable techniques.

For example, electrophysiological techniques may be used. In one such technique, a Xenopus oocyte, for example, can be used to express a receptor of the present invention. The receptor can be expressed on the oocyte membrane following micro-injection into the oocyte of RNA coding for said receptor.

When a ligand binds to the receptor, it can cause a release of calcium ions either from intracellular stores, or from extracellular sources. These calcium fluxes then cause a chloride current across the cell membrane which can be measured electrophysiologically.

Such currents are discussed in Wahlestedt, C., Ann. N.Y. Acad. Sci. 632 116-22 (1991) and Boton, R *et al.*, J.

Physiol. (London) **408** 511-534 (1989), for example.

As an alternative to using electrophysiological techniques or other techniques which rely upon a biological response to receptor binding, more direct assays of binding can be used. Thus ligands could be labelled with a detectable label, allowed to bind to a receptor, and the label could then be detected. Suitable labels which could be used include radiolabels, fluorescent labels, enzymes which can cause a detectable change, etc.

The receptor of the present invention may also be used to screen for agents suitable for treating atheromas. In this regard it should be noted that MCP-1 is a key recruiter of monocytes to atherosclerotic plagues. The receptor can be used to screen for agents which prevent or reduce such recruitment (act antagonistically to MCP-1α). Such agents may be variants of MCP-1 itself (wherein one or more amino acids are deleted, substituted or inserted relative to MCP-1), although this is not essential.

A further use of the receptor of the present invention is to screen for agents which cause inhibition of stem cell proliferation, in other words to screen for agonists of MIP-1α. MIP-1α has been shown (Graham, G.J. *et al.*, Nature **344** 442- (1990)) to inhibit proliferation of hemopoetic stem cell proliferation. As such, receptor agonists could be used to prevent stem cell proliferation during chemotherapy, which would therefore protect the stem cells from the potentially damaging effects of such chemotherapy.

MIP-1α is known to be a stem cell proliferation inhibitor

and agents which are also stem cell proliferation inhibitors can be screened using the receptor of the present invention. Such agents may be variants of MIP-1α itself (wherein one or more amino acids are deleted, substituted or inserted relative to MIP-1α), although this is not essential.

Another use of the receptor of the present invention is in screening for agents useful in reducing the likelihood of transplant rejection or in increasing the length of time before rejection occurs. High levels of RANTES are sometimes found in renal grafts and may be associated with the rejection of such grafts. Agents which prevent or reduce the binding of RANTES to the receptor of the present invention may therefore be useful in transplantation by acting antagonistically to RANTES. Such agents may be variants of RANTES itself (wherein one or more amino acids are deleted, substituted or inserted relative to RANTES), although this is not essential.

A further use of the present invention is in screening for substances useful in treating diseases mediated by viruses. Thus it may be used as a screen for antiviral agents.

One example of this is in screening for agents useful in treating AIDS. MIP-1α and RANTES levels have been suggested as being at least partially responsible for certain AIDS patients surviving longer than others. Since a receptor of the present invention may bind to MIP-1α and/or RANTES, it can be used for screening for other agents which could be useful in treating AIDS.

It is also notable that Human Cytomegalovirus and Herpes viruses have chemokine receptors. The present invention

could be used to screen for agents useful in treating diseases mediated by such viruses.

It should be noted that the present invention is not limited to the receptor having the amino acids sequence shown in Fig 3 but that it covers variants (allelic and non-allelic variants) having one or more amino acid deletions, insertions or substitutions relative to said sequence, provided that said variants are capable of binding to at least one of the chemokines: RANTES, MIP-1α and MCP-1. (Desirably, however, the receptors are capable of binding to all of these chemokines). Binding may be determined by monitoring the response of cells in electrophysiological assay using oocytes, as already described.

For example, it will be appreciated by the skilled person that various amino acids may often be substituted for other amino acids which have similar properties without substantially altering or adversely affecting certain properties of a protein. Thus the amino acids glycine, valine, leucine or isoleucine can often be substituted for one another (amino acids having aliphatic hydroxyl side chains). Other amino acids which can often be substituted for one another include:
phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains); lysine, arginine and histidine (amino acids having basic side chains); aspartate and glutamate (amino acids having acidic side chains); asparagine and glutamine (amino acids having amide side chains) and cysteine and methionine (amino acids having sulphur containing side chains). Thus the present invention includes within its scope variants of the receptor shown in Fig 3 which includes one or more such substitutions.

It is however preferred that variants of the receptor having the amino acid sequence shown in Fig 3 have substantial amino acid identity with said amino acid sequence. The degree of amino acid identity can be calculated using a program such as "bestfit" (Smith and Waterman, Advances in Applied Mathematics, 482-489 (1981)) to find the best segment of similarity between the two sequences. The alignment is based on maximising the score achieved using a matrix of amino acid similarities, such as that described by Schwarz and Dayhof (1979) Atlas of Protein Sequence and Structure, Dayhof, M.O., Ed pp 353-358.

Preferably however the degree of sequence identity is at least 50% or at least 60%, and more preferably it is above 75%. Sequence identities of at least 80%, e.g. at least 90% or at least 95%, are most preferred.

The receptor or variant thereof may include an N-terminal methionine. Such methionines are sometimes incorporated during translation and not subsequently removed.

The receptor or variant may be covalently linked to another moiety (e.g. a protein). Thus fusion proteins may be formed. These are well known in the art and may be used to assist in identification or purification or to otherwise alter the properties of the receptor of a variant thereof (e.g. to alter its stability and/or is binding properties).

Truncated variants of the receptor having the amino acid sequence shown in Figure 3 may also be provided since one or more amino acids may be deleted from said sequence, whilst retaining binding to MIP-1α, RANTES and/or MCP-1. These may be N-terminal deletions, C-terminal deletions

or may occur within said sequence.

The receptor or variant (of whatever nature) may be provided in substantially pure form. It may be isolated from other proteins and may be isolated from a cell membrane. It may be in glycosylated or unglycosylated form (depending upon the expression system used). A receptor or variant thereof of the present invention may be provided by any appropriate technique.

Gene cloning techniques are preferably used. Such techniques are disclosed, for example, in J. Sambrook *et al.*, *Molecular Cloning* 2nd Edition, Cold Spring Harbor Laboratory Press (1989).

Alternatively, chemical synthesis may be used (although this is less preferred). For example, short synthetic peptides may be prepared and then linked together to provide a substance of the present invention. Such peptides can be prepared by techniques known to those skilled in the art. Thus one end of a molecule can be immobilised and desired amino acid residue can be added sequentially. Protective groups can be used to avoid undesired side-reactions and may then be removed.

Variants of the receptor of the present invention together with the receptor itself are referred to below as substances of the present invention.

Such substances can be used in raising or selecting antibodies. The present invention therefore includes antibodies which bind to a substance of the present invention. Preferred antibodies bind specifically to substances of the present invention so that they can be used to purify such substances. The antibodies may be

monoclonal or polyclonal.

Polyclonal antibodies can be raised by stimulating their production in a suitable animal host (e.g. a mouse, rat, guinea pig, rabbit, sheep, goat or monkey) when the substance of the present invention is injected into the animal. If necessary an adjuvant may be administered together with the substance of the present invention. The antibodies can then be purified by virtue of their binding to a substance of the present invention.

Monoclonal antibodies can be produced from hybridomas. These can be formed by fusing myeloma cells and spleen cells which produce the desired antibody in order to form an immortal cell line. This is the well known Kohler & Milstein technique (*Nature* **256** 52-55 (1975)).

Techniques for producing monoclonal and polyclonal antibodies which bind to a particular protein are now well developed in the art. They are discussed in standard immunology textbooks, for example in Roitt *et al*, *Immunology* second edition (1989), Churchill Livingstone, London.

In addition to whole antibodies, the present invention includes derivatives thereof which are capable of binding to substances of the present invention.

Thus the present invention includes antibody fragments and synthetic constructs. Examples of antibody fragments and synthetic constructs are given by Dougall *et al* in *Tibtech* **12** 372-379 (September 1994).

Antibody fragments include, for example, Fab, $F(ab')_2$ and Fv fragments (see Roitt et al [*supra*]).

Fv fragments can be modified to produce a synthetic construct known as a single chain Fv (scFv) molecule. This includes a peptide linker covalently joining V_h and V_l regions which contribute to the stability of the molecule.

Other synthetic constructs include CDR peptides. These are synthetic peptides comprising antigen binding determinants. Peptide mimetics may also be used. These molecules are usually conformationally restricted organic rings which mimic the structure of a CDR loop and which include antigen-interactive side chains.

Synthetic constructs include chimaeric molecules. Thus, for example, humanised (or primatised) antibodies or derivatives thereof are within the scope of the present invention. An example of a humanised antibody is an antibody having human framework regions, but rodent hypervariable regions.

Synthetic constructs also include molecules comprising a covalently linked moiety which provides the molecule with some desirable property in addition to antigen binding. For example the moiety may be a label (e.g. a fluorescent or radioactive label) or a pharmaceutically active agent.

The antibodies or derivatives thereof of the present invention have a wide variety of uses. They can be used in purification and/or identification of the substances of the present invention. Thus they may be used in diagnosis.

They can be provided in the form of a kit for screening for the substances of the present invention.

The present invention also includes within its scope nucleic acid molecules coding for substances of the present invention (i.e. for the aforesaid receptor or variants thereof). The nucleic acid molecules may be RNA or DNA and may be provided in isolated or recombinant form.

Nucleic acid molecules of the present invention may be provided by any appropriate technique.

Gene cloning techniques are preferred (see Sambrook *et al, supra*). Variants of a given nucleic acid sequence can be prepared by mutagenesis techniques (e.g. site directed mutagenesis).

Chemical synthesis techniques can alternatively be used, but are less preferred.

Vectors may be used to incorporate the nucleic acid molecules of the present invention. The vectors may be eukaryotic or prokaryotic vectors and may be incorporated into appropriate host cells or into non-cellular expression systems.

Nucleic acid molecules which are complementary to the aforesaid nucleic acid molecules are also within the scope of the present invention. These are sometimes referred to as "antisense molecules". They can hybridise to complementary nucleic acid molecules and may thereby prevent or reduce the expression of a gene product. Thus they can be used to alter gene expression.

The use of such molecules is useful in studying gene function and regulation. Appropriate labelling and hybridisation techniques can be useful to identify the

location of coding regions.

The present invention also includes within its scope nucleic acids which can be used as probes for chemokine receptors. Preferred probes can hybridise specifically to a nucleic acid coding for the protein having the amino acid sequences given in Fig 3, or for variants thereof, as described above. Such probes can be of any suitable length, but would typically be above 15 nucleotides long and may be at least 100 nucleotides long

Desirably probes will hybridise to a target sequence under stringent hybridisation conditions. An example of stringent hybridisation conditions is a temperature of 35°-65°C and a salt concentration of about 0.9 molar. Other stringent hybridisation conditions may be used and the salt concentration need not be as high as 0.9 molar.

The nucleic acid sequences given in Figs 1 and 3 herein or fragments thereof can be used as probes or primers or to prepare probes or primers.

The primers may be used to amplify nucleic acid sequences e.g. by using PCR or other amplification techniques. The probes may be used in diagnosis or in purification.

The present invention will now be explained by way of example only, with reference to the accompanying drawings, wherein:

Fig 1 shows a cDNA sequence and a deduced amino acid sequence of a clone designated "TM(2-7)5.5", which was used to probe a human spleen λGT11 cDNA library.

Fig 2 shows various primers which were used in sequencing

a clone isolated from the library referred to in respect of Fig 1 above by using the TM(2-7)5.5 DNA as a probe.

Fig 3 shows the cDNA sequence and the deduced amino acid sequence in respect of the clone referred to in respect of Fig 2 above, the clone being designated "K5.5".

Figs 4 to 6 show Northern Blot analyses prepared using TM(2-7)5.5 DNA in various hybridisation studies. In these figures the following scoring system is used:

+++ Very strong positive signal visible after four hours' exposure of the autoradiograph.

++ Clear positive signal visible after four hours' exposure of the autoradiograph.

+ Signal not visible after four hours' exposure of the autoradiograph, but clear after 24 hours.

+/- Weak positive signal only visible after 24 hours' exposure.

- No signal.

Probes were used at a specific activity of 10^6 cpm/ml hybridisation solution.

Fig 7 shows on agarose gels an analysis of K5.5 receptor mRNA expression products from leukocytes and some human cell lines, RNA having been amplified using reverse transcriptase PCR.

Fig 8A shows an analysis of the current induced in

voltage clamped Xenopus oocytes, into which K5.5 cRNA had been micro-injected, on stimulation with various chemokine ligands.

Fig 8B shows an analysis similar to that performed in respect of Fig. 8A but using different chemokines (apart from MIP-1α, which is shown in both figures for comparison). The present inventors were unable to obtain any data showing that IL-8 binds to CC-CKR3 molecules. Preferred receptors within the scope of the present invention do not bind to IL-8.

Fig 9 shows the results of a binding assay using [^{125}I] MIP-1α and [^{125}I] RANTES to bind to human and murine CC-CKR-3 molecules.

Examples

An alignment of the amino acid sequences of IL-8 receptor A and B and of the C-C CKR-1(MIP-1α/RANTES receptor) indicated that a region between proposed transmembrane domains 3 and 4 contains the conserved amino acid sequence R Y L A I V H A.

A second conserved amino acid sequence occurs in the proposed 7th transmembrane domain in these three receptors as well as in two non-chemokine chemotactic peptide receptors for fMLP (formyl-methionine-leucine-phenylalanine) and C5a as follows:

C L(or V,I) N P I(or L,M,V) I(or L) Y A(or V) F(or V)

Degenerate oligonucleotides were prepared containing the majority of possible codons which could be translated to give the above-mentioned amino acid sequences.

These oligonucleotides had the sequences:

a) sense 5′ GIT AYY TIG CIA THG TIC AYG C

or

b) antisense 5′ AMI RCR TAI ADI AII GGR TTI AIR C

using the IUB/GCG codes, wherein

I = inosine which can substitute for A, T, G, or C
Y = C or T
H = A, C or T
M = A or C
R = A or G
D = A, G or T

The oligonucleotides were then used to clone a human CC chemokine receptor using the procedure set out below.

(a) Cloning of a sequence designated human K5.5 (CC-CKR-3)*

(* The designation CC-CKR-3 is used here for consistency with the designation used in the priority document. However it is noted that other research groups are now using the designation CC-CKR-3 for a different molecule and that the molecule referred to herein as CC-CKR-3 may now be referred to in the literature as CC-CKR-4.)

Total RNA was isolated from 1 x 10^8 KU812 cells by the method of Chomczynski and Sacchi, (1987) (Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, Anal. Biochem. **162** 156-159). These cells were from a human basophilic KU812 cell line which was a gift of Dr K Kishi, Niigata, Japan.

PolyA+ mRNA was subsequently isolated by oligodT cellulose chromatography using a polyA quik mRNA purification kit (Stratagene). Single-stranded cDNA was prepared from 1 μg of polyA+ mRNA in a 50 μl reaction containing 1 μg oligodT_{12-18}, 4 mM methyl mercuric hydroxide, 1 mM dNTPs, 50mM Tris-HCl pH 8.3 buffer, 50 mM KCl, 8 mM $MgCl_2$, 10 units RNAsin and 100 units of AMV reverse transcriptase-XL (Life Sciences Inc.) for 60 min at 42°C. 5 μl aliquots of the reaction mixture were then subjected to 40 cycles of PCR (95°C, 2 min; 37°C, 2 min and 72°C, 2 min) in 10 mM Tris-HCl pH 8.3 buffer, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPS and 2.5 units of AmplitaqTM (Perkin Elmer Cetus) using 3 μM of each

degenerate oligonucleotide primer (sense 5' GIT AYY TIG CIA THG TIC AYG C and antisense 5' AMI RCR TAI ADI AII GGR TTI AIR C) in a Techne PHC-2 thermal cycler.

PCR reaction products were visualized on 1% agarose gels containing 0.5 μg/ml ethidium bromide. Reaction products migrating at the predicted size (500-550bp) were gel purified by standard methods (Sambrook J. *et al.*, 1989 in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY). Gel purified DNA was then rendered blunt-ended by sequential treatment with T4 polynucleotide kinase (New England Biolabs) according to the manufacturer's instructions, in a total volume of 50 μl for 1 h at 37°C. After this time, 2.5 μl of 2.5 mM dNTPs and 1 μl of *E. coli* DNA polymerase I Klenow fragment (New England Biolabs) were added and the incubation continued for a further 30 min at 37°C. The reaction mixture was then heat inactivated at 70°C for 30 min and then extracted once with Tris-HCl pH 8.0 saturated phenol/chloroform (1:1 v/v). DNA was precipitated by addition of 10 μl 3M sodium acetate pH 5.5, 1 μl glycogen (20 mg/ml) (Boehringer) and 250 μl ethanol at -20°C. The DNA was recovered by centrifugation at 10 000 x g for 20 min at 4°C and washed with 70 % ethanol. The final pellet was resuspended in sterile water at a concentration of 10 ng/μl.

A pBluescript II SK- cloning vector (Stratagene) was prepared as follows: 20 μg of CsCl gradient purified plasmid was digested in a reaction volume of 100 μl for 2 h at 37°C with 200 units of *Eco* RV or *Eco* RI (New England Biolabs) according to the manufacturer's instructions. After 2 h, the digested vector was treated with 10 μl of calf intestinal alkaline phosphatase (20 units/ml) (Boehringer) for a further 30 min at 37°C.

The reaction mixture was inactivated by heating at 68°C for 15 min and then extracted once with Tris-HCl pH 8.0 saturated phenol/chloroform (1:1 v/v). Plasmid DNA was precipitated by addition of 10 µl 3M sodium acetate pH 5.5 and 250 µl ethanol at -20°C. The DNA was recovered by centrifugation at 10 000 x g for 20 min at 4°C, washed with 70 % ethanol. The final pellet was resuspended in sterile water at a concentration of 50 ng/ml.

Blunt-ended PCR product (10 ng) was ligated to 50ng of *Eco* RV digested, alkaline phosphatase treated pBluescript II SK- plasmid cloning vector in a 20 µl volume using 2 µl of T4 DNA ligase (400 000 units/ml) (New England Biolabs) for at least 16 h at 15°C. Ligation products were diluted to 100 µl with 1 x TE (10 mM Tris-HCl pH 8.0/1 mM EDTA) and phenol/chloroform extracted as described previously. Ligation products were precipitated by the addition of 10 µl 3M sodium acetate pH 5.5, 1 µl glycogen (20 mg/ml) and 250 µl ethanol for 15 min at -70°C. DNA was recovered by centrifugation as described above and resuspended in 10 µl of sterile water. Five µl of resuspended ligation products were then electroporated into electrocompetent *E. coli* strain XL-1 blue (recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, lac, {F' proAB, lacIqZDM15, Tn10 (tet^r)] (40 µl) using a Bio Rad Gene pulser according to the manufacturer's instructions. Following electroporation, 1 ml of LB medium was added and cells were grown at 37°C for 1 h. After this time, 100 µl aliquots of the culture medium were plated on LB plates containing 100 µg/ml of ampicillin and grown up for 16 h at 37°C. Individual bacterial colonies were then picked into 5 ml of LB medium containing 100 µg/ml of ampicillin and grown overnight at 37°C. Small scale plasmid DNA preparations

(mini-preps) were then made from 3 ml of each culture using a Wizard™ mini-prep DNA purification system (Promega) according to the manufacturer's instructions. Three µl aliquots of mini-prep DNA was then digested with restriction enzymes *Hind* III and *Eco* RI (both from New England Biolabs) according to the manufacturer's instructions in a reaction volume of 15 µl. Reaction products were analysed on 1% agarose gels containing 0.5 µg/ml ethidium bromide. Mini-prep DNAs which yielded an insert size of approximately 500-550 bp were then subjected to DNA sequence analysis using T3 and T7 primers and Sequenase (USB) according to the manufacturer's instructions.

A comparison of the sequences obtained against the GenBank/EMBL/DDBJ databases revealed that 10/23 sequences analysed showed 60% identity at the DNA level to the human C-C CKR-1 (MIP-1α/RANTES receptor) (Neote *et al.*, Molecular cloning, functional expression and signalling characteristics of a C-C chemokine receptor, Cell 72 415-425 (1993)). The sequence of one of the clones designated TM(2-7)5.5 (shortened to K5.5) is shown in figure 1.

CsCl gradient-purified plasmid DNA was prepared for clone K5.5 by standard methods. 20 µg of plasmid DNA was digested at 37°C with restriction enzymes *Hind* III and *Eco* RI according to the manufacturer's instructions (New England Biolabs). Digestion products were analysed on 1% agarose gels containing 0.5 µg/ml ethidium bromide. The 514 bp insert DNA corresponding to the sequenced PCR product was gel purified as described previously. One hundred ng of the 514 bp insert was labelled with ^{32}P-dCTP (Amersham International) using a random-primed DNA-labelling kit (Boehringer) according to the

manufacturer's instructions, and used to screen 5 x 10^5 clones of a human spleen λGT11 cDNA library (Clontech) according to the manufacturer's instructions. Following hybridization, duplicating positives were rescreened with the same probe until a pure positive phage plaque was obtained. Phage DNA was recovered from positive plaques using standard methods (Sambrook J. *et al* (1989)). Purified phage DNA (100 μg) was digested with 200 units of *Eco* RI (New England Biolabs) in buffer 2 (New England Biolabs) for 16 h at 37°C. Digestion products were fractionated on 1% agarose gels containing ethidium bromide (0.5 μg/ml) and cDNA inserts were gel purified and ligated into the *Eco* RI site of pBluescript II SK- vector as described above. Ligation products were transformed into *E. coli* strain XL-1 blue (recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, lac, {F' proAB, lacIqZDM15, Tn10 (tet^r)] by electroporation as previously. Individual, ampicillin resistant bacterial colonies were inoculated into L-Broth containing 100 μg/ml ampicillin and grown up for 16 h at 37°C. Mini-prep DNA was prepared from 3 ml of overnight culture medium as described above. Three μl aliquots of mini-prep DNA was then digested with restriction enzyme *Eco* RI according to the manufacturers' instructions in a reaction volume of 15 μl. Reaction products were analysed on 1% agarose gels containing 0.5 μg/ml ethidium bromide. Mini-preps which contained cDNA inserts were subsequently sequenced using Sequenase™ and T3 and T7 primers on an Applied Biosystems DNA sequencer.

One clone designated E1-C19, was shown by sequencing with the T7 primer to contain the putative 5' end of K5.5. CsCl gradient-purified DNA of clone E1-C19 was subsequently resequenced with T3 and T7 primers and several internal sequencing primers based on the previous

sequencing results (primer sequences are shown in figure 2). The sequence of E1-C19 insert cDNA is shown in figure 3.

(b) Northern Blot Analysis

Multiple tissue Northern blots were purchased from Clontech and hybridized to the 514 bp *Hind* III/*Eco* RI fragment of pTM(2-7)5.5 according to the manufacturer's instructions. For other Northern blots, total RNA was prepared from cell lines and peripheral blood leukocyte populations by the method of Chomczynski and Sacchi (1987). All of the cell lines used in this study were maintained in RPMI 1640 medium containing 10% heat inactivated FCS and 50 μg/ml gentamycin (all purchased from Gibco-BRL). Total peripheral blood mononuclear cells and polymorphonuclear cells were purified by density gradient centrifugation on Ficoll (Pharmacia). Leukocytes were sorted by FACS using the appropriately labelled antibody on a FACS star (Becton Dickinson) to obtain pure populations (>90%) of B cells (CD20), T cells (CD4, CD8, CD45R0, CD45RA) and monocytes (CD14). Pulmonary macrophages and mixed lung leukocytes were prepared from resected human lung samples using the method of Nicod *et al* (1989) (Separation of potent and poorly functional human lung accessory cells based on autofluorescence. J. Leukocyte. Biol. 45 458).

5 μg of each RNA was electrophoresed in 1% agarose gels containing 2.2% (v/v) formaldehyde, transferred to nitrocellulose and probed with the ^{32}P-dCTP labelled 514 bp insert from TM(2-7)5.5 using standard Northern blot procedure (Sambrook *et al* (1989)). The results are shown in Figs 4 to 6.

(c) Analysis of K5.5 receptor mRNA expression in leukocytes and some human cell lines by reverse transcriptase PCR

10 μg of total RNA (in a volume of 10 μl) and 0.5 μl of 0.5 mg/ml solution of oligodT_{15} were heated at 70°C for 10 min and then cooled on ice for 2 min, followed by addition of 4 μl of 5X 1st strand buffer, 2 μl of 0.1 M DTT, 1 μl of 10 mM dNTPs and 1 μl Superscript™ for 1 h at 37°C. All reagents for the reverse transcription (RT) reaction were from Gibco-BRL except oligodT_{15} (Stratagene). Two μl aliquots of each RT reaction was then subjected to 40 cycles of PCR (2 min 95°C; 2 min, 55°C and 2 min, 72°C) in a 100 μl reaction mixture containing 100 pmoles each of primers K5-5FLA and K5-5FLB. PCR reaction products (10 μl) were analysed on 1% agarose gels as described above, for the presence of a 1085 bp reaction product corresponding to the full coding sequence of K5.5. The results are shown in Fig 7, wherein the samples in the lanes indicated in Fig 7 are as follows:

Lane	Sample
1	Molecular weight markers (1 kb ladder)
2	PB T cells (IL-2 stimulated)
3	PB T cells
4	Jurkat
5	MOLT-4
6	PB B cells
7	PB B cells
8	pulmonary macrophages
9	PB monocytes
10	KU812
11	EOL-3
12	SW900 (lung epithelial cell line)
13	CCLu32 (lung fibroblast cell line)
14	LL24 (lung fibroblast cell line)
15	AALT.16 (aortic smooth muscle cell line)

(d) Expression of K5.5 cRNA in Xenopus oocytes

CsCl-gradient purified pE1-C19 plasmid DNA (5 μg) was linearized using restriction enzyme *Bam* HI (New England Biolabs) in a 100 μl reaction volume overnight at 37°C. Linearized plasmids were treated with 2 μl of proteinase K (16.7 mg/ml Boehringer) for 30 min at 37°C. DNA was extracted twice with phenol (0.1 M Tris-saturated pH 8.0) and once with chloroform. Glycogen (1 μl of 20 mg/ml stock solution) was added to the aqueous phase and linearized DNA was precipitated following addition of 0.1 volume of 3 M sodium acetate pH 5.5 and 2.5 volumes of ethanol for 1 h at -80°C. The DNA was recovered by centrifugation (14 000 rpm, 4°C in a microfuge), washed

with 70% ethanol and dissolved in RNase free water at 250 ng/ml.

Capped cRNA transcripts were generated from 1 μg of *Bam* HI (New England Biolabs) linearized DNA in a 100 μl reaction volume containing 20 μl transcription buffer (5X), 4 μl NTP mix (10 mM ATP, UTP and CTP, 3 mM GTP), 4 μl 0.75M DTT, 2.5 μl RNAsin, 0.5 μl GTP (10 mM), 4 μl CAP analog (10 mM m7G(5')ppp(5')G) and 2.5 μl of T7 or T3 RNA polymerase respectively. All reagents used for the *in vitro* transcription reaction were from Promega except CAP analog (Pharmacia). After 1.5 h at 37°C, 4 μl RQ1 DNase (Promega) was added and the reaction mixture was incubated for a further 15 min at 37°C. The reaction mixture was extracted twice with 0.1 M Tris-HCl pH 8.0 saturated phenol/chloroform (1:1 v/v) and once with chloroform. Glycogen (1 μl as above) was added to the aqueous phase and cRNA was precipitated overnight at -20°C after addition of 0.1 volume, 3 M sodium acetate pH 5.5 and 2.5 volumes ethanol. cRNA was recovered by centrifugation (14 000 rpm, 4°C, 20 min in a microfuge), the pellet washed in 70 % ethanol and resuspended in sterile water at 1 μg/μl. An approximate estimate of the cRNA concentration was obtained by running an aliquot of the resuspended material on a 1% agarose gel containing 2.2% (v/v) formaldehyde against RNA markers of known concentration. Samples were stored at -80°C before use.

Oocytes were harvested from adult female Xenopus laevis, by standard methods (Bertrand *et al.*, 1991). Oocytes were defollicullated by incubation in 0.2% (w/v) collagenase (Sigma) in 50 ml OR2 medium without Ca2+ and without Mg2+ in a spinner flask under slow agitation for 2 h at room temperature (OR2 medium is 82.5 mM NaCl, 2.5 mM KCl, 1 mM Na_2HPO_4, 15 mM HEPES, 2 mM $CaCl_2$, 1 mM

$MgCl_2$ pH 7.6). Oocytes were rinsed carefully with OR2 followed by MBS (modified Barth's saline: 88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM HEPES, pH 7.6) and allowed to recover for at least 1-2 h in MBS before selecting stage V-VI oocytes. Selected oocytes were incubated in MBS supplemented penicillin/streptomycin (100 units/ml) (Gibco-BRL) overnight at 18°C before injection.

Oocytes were microinjected using an Inject + Matic air pump (Gabay) using needles made from Drummond calibrated 6 ml capillaries. cRNA (25 ng in 50 nl) was injected into the cytoplasm. Oocytes were individually transferred to wells of a 96 well flat bottom culture dish and incubated in MBS for 24-72 h.

Electrophysiological recordings were made 1-3 days after injection in oocytes superfused with OR2 medium at room temperature under voltage clamped conditions using two microelectrodes (1-2 MΩ, both filled with 3 M KCl), the membrane potential being routinely clamped at -100 mV using a Gene Clamp 500 instrument (Axon).

Test chemokines were purchased from PeptroTech or produced in-house at the Glaxo Institute for Molecular Biology and resuspended at a concentration of 1 μM in PBS. Fifty μl of each chemokine was applied directly onto voltage clamped oocytes and the current induced was monitored on a Tektronix 5113 dual-beam storage oscilloscope linked to an IBM-PC. Where multiple chemokines were tested on a single oocyte, a recovery time of 2 min was allowed between each application. The results are shown in Fig 8A.

Fig 8B shows the results of a similar analysis to that

illustrated in Fig 8A, but using different chemokines (apart from MIP-1α).

It can be seen that no significant electrophysiological response was seen when using IL-8, in contrast with the result obtained for MIP-1α.

(e) HL-60 Cell Transfection and Ligand Binding Assay

Thirty μg human CC-CKR-3-pcDNA1neo, murine CC-CKR-3-pcDNA1neo, or pcDNA1neo were electroporated into 500 μl HL-60 cells (2 x 10^7 cells/ml in 0.15 M NaCl, 20mM HEPES, pH 7.3) using a Bio Rad Geno Pulster (260 volts, 960 μF, 0.4 cm gap cuvette). Cells were seeded into T-175 flasks containing 25 ml AIM-V serum-free media (GIBCO). On day 2 or 3 following transfection the cells were diluted in a total volume of 45 ml AIM-V media containing 600 μg/ml G418, and on day 6, cells were further diluted to 180 ml AIM-V media containing 600 μg/ml G418. On days 7-15 post-transfection cells were maintained in AIM-V media (+G418) at a density of 0.4 - 1.2x 10^6 cells/ml, and binding assays were performed during this time. Equilibrium competition binding was carried out by incubating 5 x 10_5 cells in 100 μl binding buffer (1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% BSA, 50 mM HEPES, pH 7.2), 0.34 nM [^{125}I]radioligand, and 0.5 - 2000 nM cold ligand in Millipore®-DV96-well filter plates. After 1.5h incubation at room temperature, cells were washed four times by vacuum filtration with binding buffer containing 0.5 M NaCl. Fifty μl Optiphase scintillant (Wallac) were added to each well, and the radioactivity was measured with a Wallac Microbeta Plate Reader. All binding data was normalized as the percentage of total binding. Total binding for a given ligand was defined as the

radioactivity bound in the absence of competing ligand to 5 x 10^5 cells transfected with human CC-CKR-3 (range: 1000-2500 cpm).

The results are shown in Figure 9 which illustrates high affinity binding of [^{125}I]MIP-1α and [^{125}I]RANTES to human and murine CC-CKR-3. HL-60 cells were transfected with human CC-CKR-3 (●), murine CC-CKR-3 (■), or an empty vector (O) and maintained in AIM-V media containing G418 for 7-15 days. Equilibrium competition assays were performed as described above with [^{125}I]MIP-1α (A) and [^{125}I]RANTES (B). Each point represents the mean ± S.D. of duplicate points from four (A) or three (B) separate experiments. Data were curve-fitted with GraFit 3.01 software (Leatherbarrow., R.J., GraFit Versions 3.01, Erithicus Softward Ltd., Staines, UK (1992)) using the equation $B/Bmax^{app} = 1/(1+([L[/IC_{50}))$, where B = cpm bound, $Bmax^{app}$ = cpm bound in the absence of competing ligand, L = competing ligand, and the IC_{50} = [radioligand] + K_d (Cheng., Y. and Prusoff., W.H. *Biochem Pharmacol* **22**: 3099-3108 (1973)).

CLAIMS

1. A substance which:

a) has the amino acid sequence shown in Figure 3

or

b) has one or more amino acid deletions, insertions or substitutions relative to the amino acid sequence shown in Figure 3.

2. A substance according to claim 1 which is capable of binding to RANTES, MIP-1α and/or MCP-1.

3. A substance according to claim 1 or claim 2 which has substantial amino acid sequence identity with the amino acid sequence shown in Figure 3.

4. The use of a substance according to any of claims 1 to 3 in screening for an agent useful in treating allergies.

5. The use of a substance according to any of claims 1 to 3 in screening for an agent useful in treating asthma, hay fever, eczema, food allergies, atopic dermatitis, rhinitis or conjunctivitis.

6. The use of a substance according to any of claims 1 to 3 in screening for an agent which blocks the binding of MCP-1, MIP-1α and/or RANTES to a chemokine receptor.

7. The use of a substance according to any of claims 1 to 3 in screening for an agent which is involved in activating T-lymphocytes.

8. The use of a substance according to any of claims 1

to 3 in screening for an agent useful in treating atheromas.

9. The use of a substance according to any of claims 1 to 3 in screening for an agent which inhibits stem cell proliferation.

10. The use of a substance according to any of claims 1 to 3 in screening for an agent which provides some protection for stem cells against potentially damaging effects of chemotherapy.

11. The use of a substance according to any of claims 1 to 3 in screening for an agent which is useful in reducing the likelihood of transplant rejection or in increasing the length of time before rejection occurs.

12. The use of a substance according to any of claims 1 to 3 in screening for an agent useful in treating a disease mediated by viruses.

13. The use of a substance according to any of claims 1 to 3 in screening for an agent useful in treating AIDS or in treating a disease mediated by Human Cytomegalovirus or by a Herpes virus.

14. An agent which has been screened as described in any of claims 4 to 13.

15. An agent according to claim 14 for use in medicine.

16. A pharmaceutical composition comprising an agent according to claim 14 and a pharmaceutically acceptable carrier.

17. An antibody or a derivative thereof which binds to a substance according to any of claims 1 to 3.

18. A nucleic acid molecule which:

a) codes for a substance according to any of claims 1 to 3.

b) is complementary to a molecule as defined in a) above.

or

c) hybridises to a molecule as defined in a) or b) above.

19. A vector comprising a nucleic acid molecule according to claim 18.

20. A host comprising a vector according to claim 19.

21. A method for obtaining a substance according to any of claims 1 to 3, comprising incubating a host according to claim 20 under conditions causing expression of said substance and then purifying said substance.

22. A substance, use, agent, composition, antibody or derivative thereof, nucleic acid, vector, host or method; which is substantially as hereinbefore described with reference to the accompanying examples.

FIG. 1

cDNA sequence and deduced amino acid equence of clone TM(2-7)5.5

```
   1 GGTATCTGGCGATCGTGCACGCGGTCTTTTCCTTGAGGGCAAGGACCTTGACTTATGGGG   60
       Y  L  A  I  V  H  A  V  F  S  L  R  A  R  T  L  T  Y  G  V

  61 TCATCACCAGTTTGGCTACATGGTCAGTGGCTGTGTTCGCCTCCCTTCCTGGCTTTCTGT  120
       I  T  S  L  A  T  W  S  V  A  V  F  A  S  L  P  G  F  L  F

 121 TCAGCACTTGTTATACTGAGCGCAACCATACCTACTGCAAAACCAAGTACTCTCTCAACT  180
       S  T  C  Y  T  E  R  N  H  T  Y  C  K  T  K  Y  S  L  N  S

 181 CCACGACGTGGAAGGTTCTCAGCTCCCTGGAAANCAACATTCTCGGATTGGTGATCCCCT  240
       T  T  W  K  V  L  S  S  L  E  X  N  I  L  G  L  V  I  P  L

 241 TAGGGATCATGCTGTTTTGCTACTCCATGATCATCAGGACCTTGCAGCATTGTAAAAATG  300
       G  I  M  L  F  C  Y  S  M  I  I  R  T  L  Q  H  C  K  N  E

 301 AGAAGAAGAACAAGGCGGTGAAGATGATCTTTGCCGTGGTGGTCCTCTTCCTTGGGTTCT  360
       K  K  N  K  A  V  K  M  I  F  A  V  V  V  L  F  L  G  F  W

 361 GGACACCTTACAACATAGTGCTCTTCCTAGAGACCCTGGTGGAGCTAGAAGTCCTTCAGG  420
       T  P  Y  N  I  V  L  F  L  E  T  L  V  E  L  E  V  L  Q  D

 421 ACTGCACCTTTGAAAGATACTTGGACTATGCCAGCCAGGCCACAGAAACTCTGGCTTTTG  480
       C  T  F  E  R  Y  L  D  Y  A  S  Q  A  T  E  T  L  A  F  V

 481 TTCACTGCTGCCTCAATCCCCTCCTCTACGCCGT  514
       H  C  C  L  N  P  L  L  Y  A
```

FIG. 2

Oligonucleotide primers used to determine the full sequence of E1-C19

K5-5AS	5' AGA GTA CTT GGT TTT GCA GTA G		(ANTISENSE)
K5-5AS2	5' GCA GCA GTG AAC AAA AGC CAG		(ANTISENSE)
K5-5A	5' CAT AGT GCT CTT CCT AGA GAC		(SENSE)
K5-5B	5' GGT TGA GCA GGT ACA CAT CAG		(ANTISENSE)
K5-5C	5' CAA TAC TGT GGG CTC CTC C		(SENSE)
K5-5D	5' GCT CAG GTC CAT GAC TG		(SENSE)
K5-5E	5' CTC ATG AGC ATT GAT AG		(SENSE)
K5-5F	5' CTG AGC GCA ACC ATA CC		(SENSE)
K5-5G	5' GCT AGA AGT CCT TCA GG		(SENSE)
K5-5H	5' GGA TCA TGA TCT TCA TG		(SENSE)
K5-5FLA	5' AAA TGA AAC CCC ACG GAT ATA GCAG		(SENSE)
K5-5FLB	5' TCC TAC AGA GCA TCA TGA AGA TC		(ANTISENSE)

FIG. 3

cDNA sequence and deduced amino acid sequence of K5.5

```
   1 CGGGGGTTTTGATCTTCTTCCCCTTCTTTTCTTCCCCTTCTTCTTTCCTTCCTCCCTCCC  60

  61 TCTCTCATTTCCCTTCTCCTTCTCCCTCAGTCTCCACATTCAACATTGACAAGTCCATTC 120

 121 AGAAAAGCAAGCTGCTTCTGGTTGGGCCCAGACCTGCCTTGAGGAGCCTGTAGAGTTAAA 180

 181 AAATGAACCCCACGGATATAGCAGATACCACCCTCGATGAAAGCATATACAGCAATTACT 240
       M  N  P  T  D  I  A  D  T  T  L  D  E  S  I  Y  S  N  Y  Y

 241 ATCTGTATGAAAGTATCCCCAAGCCTTGCACCAAAGAAGGCATCAAGGCATTTGGGGAGC 300
       L  Y  E  S  I  P  K  P  C  T  K  E  G  I  K  A  F  G  E  L

 301 TCTTCCTGCCCCCACTGTATTCCTTGGTTTTTGTATTTGGTCTGCTTGGAAATTCTGTGG 360
       F  L  P  P  L  Y  S  L  V  F  V  F  G  L  L  G  N  S  V  V

 361 TGGTTCTGGTCCTGTTCAAATACAAGCGGCTCAGGTCCATGACTGATGTGTACCTGCTCA 420
       V  L  V  L  F  K  Y  K  R  L  R  S  M  T  D  V  Y  L  L  N

 421 ACCTTGCCATCTCGGATCTGCTCTTCGTGTTTTCCCTCCCTTTTTGGGGCTACTATGCAG 480
       L  A  I  S  D  L  L  F  V  F  S  L  P  F  W  G  Y  Y  A  A

 481 CAGACCAGTGGGTTTTTGGGCTAGGTCTGTGCAAGATGATTTCCTGGATGTACTTGGTGG 540
       D  Q  W  V  F  G  L  G  L  C  K  M  I  S  W  M  Y  L  V  G

 541 GCTTTTACAGTGGCATATTCTTTGTCATGCTCATGAGCATTGATAGATACCTGGCGATAG 600
       F  Y  S  G  I  F  F  V  M  L  M  S  I  D  R  Y  L  A  I  V

 601 TGCACGCGGTGTTTTCCTTGAGGGCAAGGACCTTGACTTATGGGGTCATCACCAGTTTGG 660
       H  A  V  F  S  L  R  A  R  T  L  T  Y  G  V  I  T  S  L  A

 661 CTACATGGTCAGTGGCTGTGTTCGCCTCCCTTCCTGGCTTTCTGTTCAGCACTTGTTATA 720
       T  W  S  V  A  V  F  A  S  L  P  G  F  L  F  S  T  C  Y  T

 721 CTGAGCGCAACCATACCTACTGCAAAACCAAGTACTCTCTCAACTCCACGACGTGGAAGG 780
       E  R  N  H  T  Y  C  K  T  K  Y  S  L  N  S  T  T  W  K  V

 781 TTCTCAGCTCCCTGGAAATCAACATTCTCGGATTGGTGATCCCCTTAGGGATCATGCTGT 840
       L  S  S  L  E  I  N  I  L  G  L  V  I  P  L  G  I  M  L  F

 841 TTTGCTACTCCATGATGATCAGGACCTTGCAGCATTGTAAAAATGAGAAGAAGAACAAGG 900
       C  Y  S  M  I  I  R  T  L  Q  H  C  K  N  E  K  K  N  K  A

 901 CGGTGAAGATGATCTTTGCCGTGGTGGTCCTCTTCCTTGGGTTCTGGACACCTTACAACA 960
       V  K  M  I  F  A  V  V  V  L  F  L  G  F  W  T  P  Y  N  I
```

```
961  TAGTGCTCTTCCTAGAGACCCTGGTGGAGCTAGAAGTCCTTCAGGACTGCACCTTTGAAA  1021
       V  L  F  L  E  T  L  V  E  L  E  V  L  Q  D  C  T  F  E  R

1021 GATACTTGGACTATGCCATCCAGGCCACAGAAACTCTGGCTTTTGTTCACTGCTGCCTTA  1080
       Y  L  D  Y  A  I  Q  A  T  E  T  L  A  F  V  H  C  C  L  N

1081 ATCCCATCATCTACTTTTTTCTGGGGGAGAAATTTCGCAAGTACATCCTACAGCTCTTCA  1140
       P  I  I  Y  F  F  L  G  E  K  F  R  K  Y  I  L  Q  L  F  K

1141 AAACCTGCAGGGGCCTTTTTGTGCTCTGCCAATACTGTGGGCTCCTCCAAATTTACTCTG  1200
       T  C  R  G  L  F  V  L  C  Q  Y  C  G  L  L  Q  I  Y  S  A

1201 CTGACACCCCCAGCTCATCTTACACGCAGTCCACCATGGATCATGATCTCCATGATGCTC  1260
       D  T  P  S  S  S  Y  T  Q  S  T  M  D  H  D  L  H  D  A  L

1261 TGTAGAAAAATGAAATGGTGAAATGCAGAGTCAATGAACTTTTCCACATTCAGAGCTTAC  1320
       *

1321 TTTAAAATTGGTATTTTTAGGTAAGAGATCCCTGAGCCAGTGGTCAGGAGGAAAGGCTTA  1380

1381 CACCCACAGGTGGGAAAGACAGGTTCTCATCCCTGCAGGNAGGTTTTTCTTCTCCCCACT  1440

1441 TAGANAAAGTNCCAGGCCTGGAAGGGGTCCAACCCNGGGTTGAGGATCCTTCCCCCAAAC  1500

1501 CCAGGGTTTGGCCTGGAGGATTAATNCAAAANNTTTNTTGAAACTCTTGAANANGTTGNG  1560

1561 NTAAGTTTNGGGGGGTTNTTTTGAAGGNAAGTTTTTCCCTTCTTNCC  1607
```

FIG. 3 CONT'D

FIG. 4

Northern blot analysis of K5.5 expression in peripheral tissue

Tissue	K5.5
heart	-
brain	-
placenta	-
lung	-
liver	-
skeletal muscle	-
kidney	-
pancreas	-
spleen	++
thymus	+++
prostate	-
testis	+/-
ovary	-
small intestine	+/-
colon	-
peripheral blood leukocytes	+++

FIG. 5

Expression of K5.5 receptor mRNA peripheral blood T cell populations and some T cell lines.

T cell/T cell lines	K5.5
T cells + IL-2 (4h)	++
" (12h)	++
" (24h)	++
" (48h)	++
" (72h)	++
" (7 days)	++
T cells + PMA (2 days)	++
Jurkat	+
MOLT - 4	+/-
T cells (unstimulated)	+
CD8 + T cell clone	+
T8 clone	+++
T8 + GH	+++
T8 + GR	-
T8 + Giα	+
T4 clone	+++
TT20 (IL2 stimulated)	++
CD45 RO	-
CD45 RO (stimulated)	-
CD45RA	-
CD45RA (stimulated)	-
HUT 78	-
HUT 78 (stimulated)	-

FIG. 6

Expression of K5.5 receptor mRNA in non-T leukocytes and cell lines

non-T cell leukocytes/lines	K5.5
peripheral blood B cells	+
B cells (activated)	
Raji	-
GCC (tonsil)	-
GCC (Anti CD40 MAb)	-
BL2 line	-
RPMI 8666	-
KU812	++
EOL-3 line	+/-
macrophages (alveolar)	-
mixed lung leukocytes	+/-
monocytes	-
HMC-1 line	-

FIG. 7

Analysis of K5.5 receptor mRNA expression by RT-PCR in human cell lines and peripheral blood leukocyte populations.

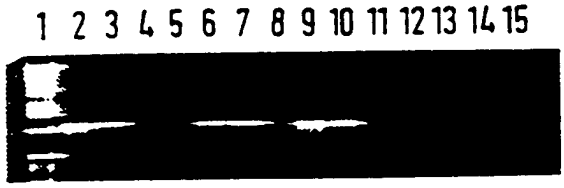


FIG. 8A

Current induced in voltage clamped oocytes on stimulation with different chemokine ligands (1 μM) at 2 min intervals (results of individual oocytes tested are shown)

K5.5

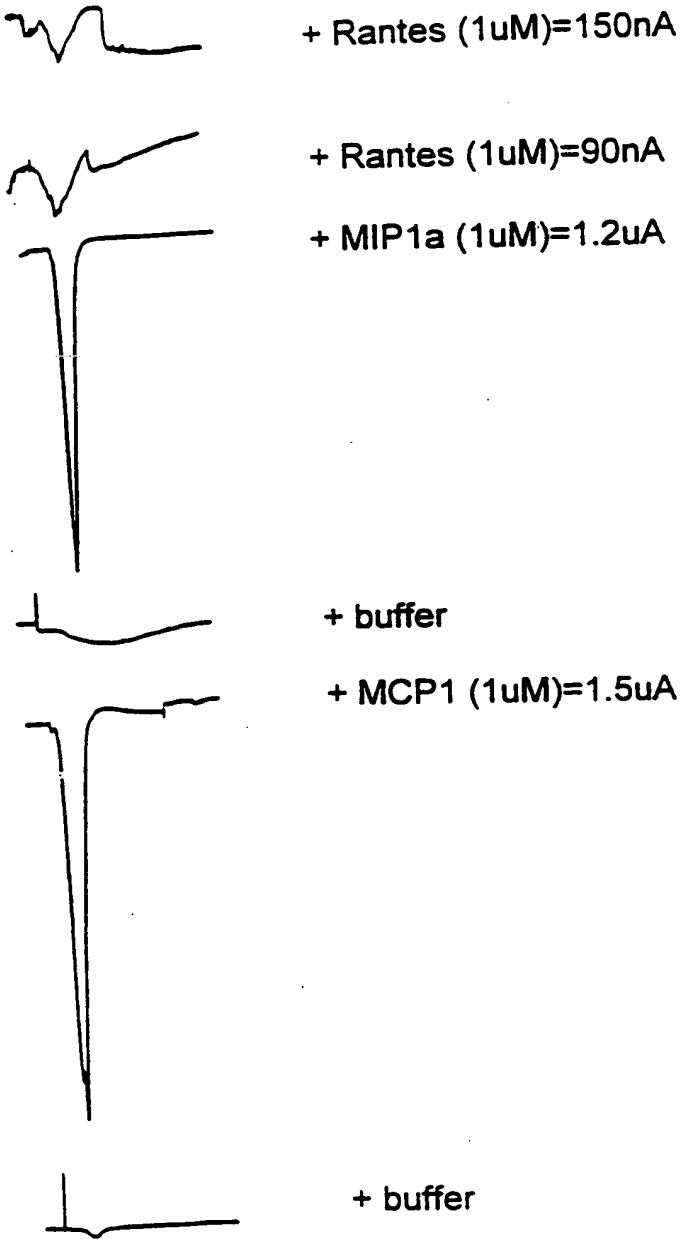
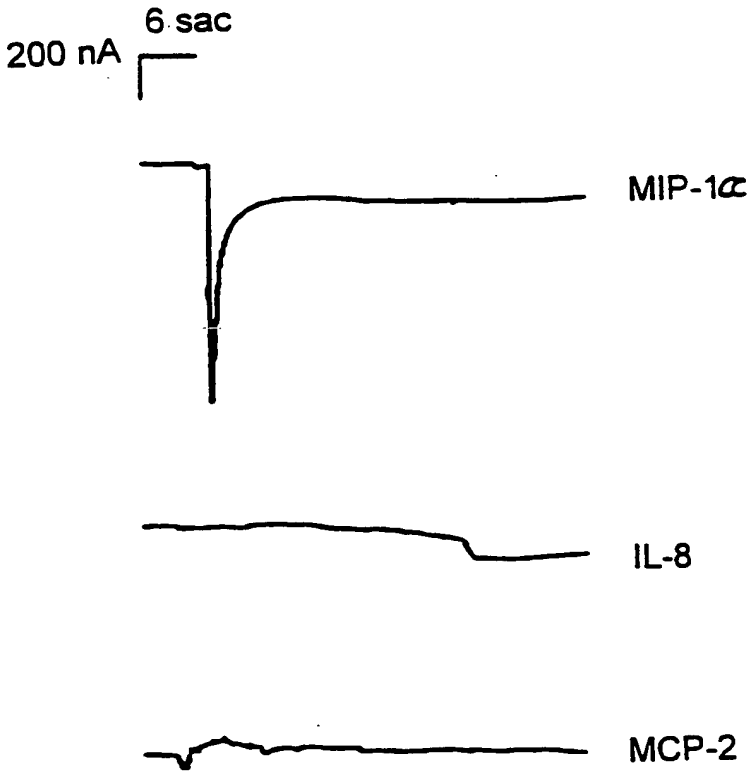


FIG. 8B



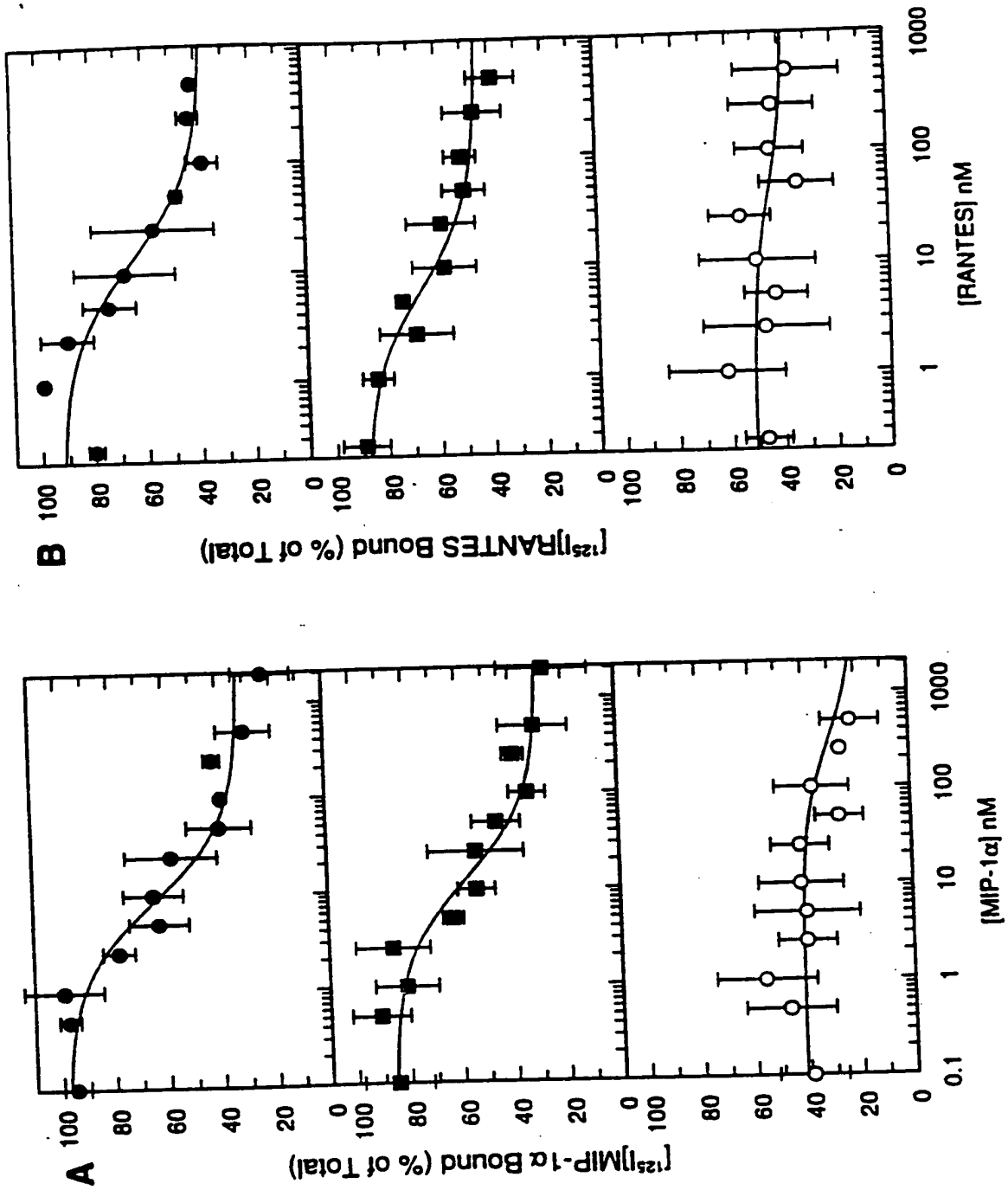

B
[125I]RANTES Bound (% of Total)
[RANTES] nM
A
[125I]MIP-1α Bound (% of Total)
[MIP-1α] nM

FIG. 9

INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/GB 96/00143

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/715 C07K16/18 G01N33/68 A61K38/17
A61K38/19 C07K14/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 23, 1993, pages 761-767, XP000567783 S.C. BISCHOFF ET AL: "RANTES and related chemokines activate human basophil granulocytes through different G protein-coupled receptors" see the whole document ---	14,22
A	WO,A,94 11504 (GENENTECH, INC.) 26 May 1994 see page 39 see claims 22,25; example II ---	1-3, 17-22
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 1996	10.05.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. al Application No
PCT/GB 96/00143

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCIENCE, vol. 244, 5 May 1989, LANCASTER, PA US, pages 569-572, XP002001324 F. LIBERT ET AL: "Selective amplification and cloning of four new members of the G protein-coupled receptor family" ---	
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, March 1994, WASHINGTON US, pages 2752-2756, XP002001325 I.F. CHARO ET AL: "Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails" see the whole document ---	1-3,14, 18-22
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 202, no. 2, 29 July 1994, ORLANDO, FL US, pages 1156-1162, XP002001326 S. YAMAGAMI ET AL: "cDNA cloning and functional expression of a human monocyte chemoattractant protein 1 receptor" see the whole document ---	1-3,14, 15,22
A	CELL, vol. 72, 12 February 1993, NA US, pages 415-425, XP002001327 K. NEOTE ET AL: "Molecular cloning, functional characteristics of a CC chemokine receptor" cited in the application see the whole document ---	1-3,14, 15,18-22
X	WO,A,94 07521 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 14 April 1994 see abstract; claims ---	14-16,22
X	WO,A,92 20372 (DANA FARBER CANCER INSTITUTE) 26 November 1992 see abstract see claims ---	14-16,22
X	WO,A,94 21277 (THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK) 29 September 1994 see abstract see claims ---	14-16,22

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 33, 18 August 1995, MD US, pages 19495-19500, XP002001328 C.A. POWER ET AL: "Molecular cloning and functional expression of a novel CC chemokine receptor cDNA from a human basophilic cell line" see the whole document -----	1-3,14, 18-22

INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB96/00143

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 4-13
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 4-13 are directed to a method of treatment of (diagnostic method practised on) the human/animal body (Rule 39.1(iv) PCT), the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

nformation on patent family members

Int onal Application No
PCT/GB 96/00143

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9411504	26-05-94	EP-A-	0669979	06-09-95
WO-A-9407521	14-04-94	AU-B-	5293293	26-04-94
WO-A-9220372	26-11-92	US-A-	5179078	12-01-93
		CA-A-	2109315	17-11-92
		EP-A-	0585337	09-03-94
		JP-T-	6508117	14-09-94
WO-A-9421277	29-09-94	US-A-	5474983	12-12-95
		AU-B-	7628094	11-10-94